Immunity
Previews

Inhibitors of Apoptosis Proteins and IL-1β: A Tangled Relationship

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DOI 10.1016/j.immuni.2012.02.007

Inhibitors of apoptosis proteins (IAPs) are important regulators of both cell death and inflammation. In this issue of *Immunity*, Vince et al. (2012) report that inhibition of IAPs results in the processing and secretion of IL-1β through RIP3-mediated caspase-1- and caspase-8-dependent pathways.

The inhibitors of apoptosis proteins (IAPs), such as XIAP, cIAP1, and cIAP2, play critical functions in regulating both apoptotic cell death and inflammatory pathways. XIAP, cIAP1, and cIAP2 contain three N-terminal baculovirus IAP repeat (BIR) domains, an ubiquitin-associated domain, and a C-terminal RING domain that possesses ubiquitin-E3 ligase activity (Lopez and Meier, 2010). In addition, cIAP1 and cIAP2 also contain a central caspase recruitment domain (CARD), which provides autoinhibition of their E3 ligase activity. Mammalian XIAP can effectively inhibit caspase-3, -7, and -9 through direct interaction. In contrast to XIAP, cIAP1 and cIAP2 are inefficient at directly inhibiting caspase activity (Broemer and Meier, 2009). cIAP1 and cIAP2 are, however, capable of promoting NF-κB activation and prosurvival signals by K63-linked polyubiquitination of receptor interacting protein 1 (RIP1) after engagement of tumor necrosis factor receptor 1 (TNFR1) by TNF-α. cIAP1 and cIAP2 also function to regulate the noncanonical NF-κB pathway by inducing the K48-linked degradative ubiquitination of the NF-κB-inducing kinase (NIK) (Lopez and Meier, 2010).

In addition to their role in regulating cell survival, IAPs also have important functions in innate immunity. Similar to the role of cIAP1 and cIAP2 in the noncanonical NF-κB pathway, cIAP1 and cIAP2 regulate the MyD88-dependent activation of MAP kinase signaling and the production of proinflammatory cytokines after Toll-like receptor 4 (TLR4) activation by mediating the degradative K48-linked polyubiquitination of TRAF3 (Tseng et al., 2010). cIAPs regulate antiviral inflammatory responses driven by the cytosolic dsRNA receptor RIG-I, again by targeting TRAF3 for degradative ubiquitination (Mao et al., 2010). IAPs are also required for proinflammatory cytokine production by the NLR family members NOD1 and NOD2 through nondegradative ubiquitination of RIP2 (Bertrand et al., 2009). The precise mechanism of XIAP modulation of NOD signaling remains unclear (Krieg et al., 2009). In this issue of *Immunity*, Vince et al. (2012) demonstrate that inhibition of XIAP, cIAP1, and cIAP2 results in the generation of IL-1β by both the NLRP3-caspase-1 inflammasome and the activation of caspase-8. Interestingly, these studies uncovered a unique role for RIP3 in this process.

To determine whether IAPs exert an effect on caspase-1 activation and IL-1β processing and secretion, the authors first employed the use of the smac-mimetic compound A. Smac mimetics are synthetic antagonists of IAPs based on the structure of the endogenous IAP inhibitor Smac (second mitochondrial activator of caspases; also known as Diablo); compound A promotes the rapid degradation of cIAP1 and cIAP2 and also antagonizes XIAP. Treatment of murine bone marrow-derived macrophages and dendritic cells, after priming with either TLR agonists or TNF-α, with compound A resulted in the activation of caspase-1 with the subsequent processing and secretion of IL-1β. Consistent with these findings, macrophages genetically deficient for XIAP, cIAP1, and cIAP2 efficiently activated caspase-1 in response to LPS-priming alone. Importantly, the inhibition of all three IAPs was necessary for achieving enhanced caspase-1 activity. Furthermore, use of macrophages deficient in components of the NLRP3 inflammasome demonstrated that compound A-induced IL-1β processing and secretion was only partially dependent on caspase-1 and the NLRP3 inflammasome.

Given that compound A-induced IL-1β processing and secretion was only partially dependent on the NLRP3 inflammasome, Vince et al. (2012) examined whether RIP1 or RIP3 were involved in this process. Depletion of IAPs has recently been shown to induce the formation of a RIP1-FADD-caspase-8-cFLIP complex termed the "ripoptosome" (Feoktistova et al., 2011; Tenev et al., 2011). The ripoptosome can trigger RIP3 signaling as well as activate caspase-8. The use of RIP3-deficient macrophages showed that both caspase-1-dependent and caspase-1-independent IL-1β secretion in response to compound A are dependent upon the presence of RIP3. However, the regulation of NLRP3 inflammasome activation by RIP3 was restricted to the smac-mimetic compound A given that other NLRP3 agonists, such as ATP and Alum, were fully capable of activating caspase-1 even in the absence of RIP3. In contrast to RIP3, inhibition of RIP1 with Necrostatin-1 did not affect compound A-induced IL-1β secretion. The finding that RIP1 appears to be dispensable for compound A-induced IL-1β secretion indicates that further evaluation of the role of the ripoptosome in this process is required.

Further evaluation revealed that the caspase-1-independent IL-1β secretion induced by compound A was mediated by caspase-8. Using shRNA knockdown of caspase-8 in both C57BL6 v-myc/v-raf immortalized macrophages and the

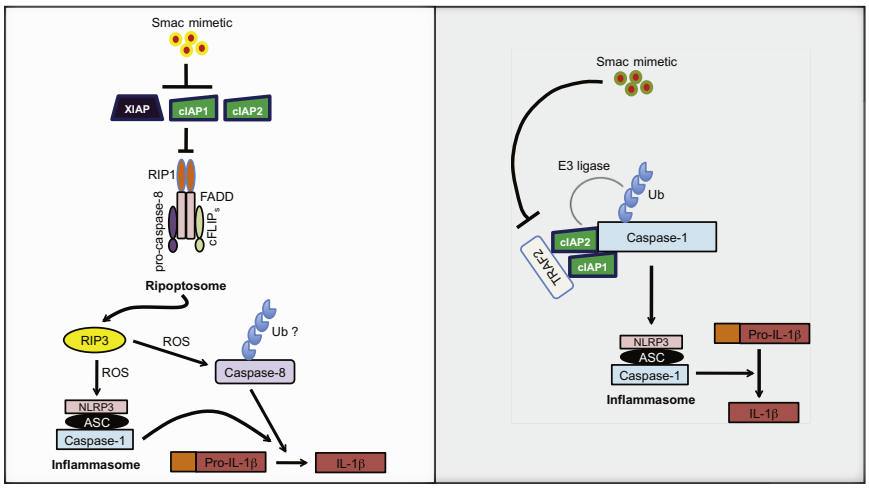

Figure 1. Positive and Negative Regulation of IL-1β Processing by IAPs
As shown on the left, Vince et al. (2012) demonstrate that inhibition of XIAP, cIAP1, and cIAP2 with the smac-mimetic compound A results in RIP3 signaling. RIP3 induces the generation of mitochondrial ROS, which is involved in activation of both the NLRP3 inflammasome as well as caspase-8. Activation of caspase-1 and caspase-8 in turn results in the processing and secretion of IL-1β. As shown on the right, Labbé et al. (2011) demonstrate that cIAP1 and cIAP2 along with TRAF2 directly interact with caspase-1 and mediate its nondegradative K63-linked polyubiquitination. Depletion of cIAP1 and cIAP2 by the smac-mimetic BV6 results in inefficient inflammasome assembly with a resultant inhibition of IL-1β secretion.

RAW264.7 murine macrophage cell line (both of which have defects in inflammasome activation), Vince et al. (2012) demonstrated that reduction of caspase-8 results in diminished IL-1β secretion in response to compound A. Previously, caspase-8 has been shown to be capable of processing pro-IL-1β into its mature 17 kDa fragment (Maelfait et al., 2008), further supporting a role for caspase-8 in the observed caspase-1-independent IL-1β maturation. The fact that RIP3-deficient macrophages have further diminished compound A-induced IL-1β secretion compared to caspase-1-deficient macrophages suggests that RIP3 plays an essential role upstream of both caspase-1 and caspase-8. Hence, it was surprising that compound A induced the processing and activation of caspase-8 even in the absence of RIP3; however, the modification of caspase-8 by compound A did appear to involve RIP3. Although ubiquitination of caspase-8 has been suggested to be required for optimal caspase-8 activity, additional studies are required to define the nature of the RIP3-mediated modification of caspase-8.

In a separate study in *Immunity*, Labbé et al. (2011) also examined the role of IAPs in caspase-1 activation. In marked contrast to Vince et al. (2012), this study found that deficiency for either cIAP1 or cIAP2 resulted in impaired caspase-1 activation. Using a cell-free system in which the inflammasome is spontaneously activated, Labbé et al. (2011) demonstrated that depletion of cIAP2 with either cIAP2 antibodies, with the smac-mimetic BV6, or utilizing macrophages from cIAP2 (*Birc3*)-deficient mice resulted in diminished caspase-1 activation and IL-1β processing. Consistent with these findings, siRNA-mediated depletion of cIAP1, cIAP2, or the adaptor TRAF2 likewise resulted in diminished caspase-1 activation and IL-1β processing in the cell-free system. Macrophages deficient in cIAP2 were also found to have diminished caspase-1 activation and IL-1β secretion in response to both NLRP3 and NLRC4 inflammasome agonists. Similarly cIAP1 (*Birc2*)- and cIAP2-deficient mice had blunted inflammatory responses in vivo to the NLRP3 inflammasome agonists monosodium urate and alum. Interestingly, cIAP1, cIAP2, and TRAF2 were found by immunoprecipitation to directly interact with caspase-1 and mediated its nondegradative K63-linked polyubiquitination.

Taken together, the studies by Vince et al. (2012) and Labbé et al. (2011) demonstrate that IAPs play critical roles in both positively and negatively regulating caspase-1 activation. Although cIAP1 and cIAP2 are not essential for inflammasome activation, Labbé et al. (2011) suggest that they along with TRAF2 aid in inflammasome activation possibly through providing the appropriate polyubiquitination modifications of caspase-1 that may allow it to more effectively assemble NLRP3 and NLRC4 inflammasome complexes (Figure 1). Vince et al. (2012) also show a role for IAPs in caspase-1 activation, but in contrast to Labbé et al. (2011) they found that depletion of XIAP, cIAP1, and cIAP2 leads to RIP3-dependent generation of reactive oxygen species that subsequently results in caspase-1 activation as part of the NLRP3 inflammasome (Figure 1). Clearly, further studies will be required to reconcile these two apparently discrepant roles of IAPs in caspase-1 activation. However, given the diverse roles of IAPs it may be very possible that cIAP1 and cIAP2 may be functioning directly at the level of caspase-1 modification while at the same time XIAP, cIAP1, and cIAP2 are holding RIP3 in check. Regardless of the pro- or anti-inflammatory roles of IAPs, it is essential that we better understand the inflammatory consequences of IAP inhibition by smac mimetics given their potential therapeutic usages in malignancy. The use of smac mimetics by Vince et al. (2012) has also uncovered an interesting alternate pathway leading to IL-1β processing and secretion involving RIP3 and caspase-8 (Figure 1). The finding that RIP3-driven reactive oxygen species production can induce caspase-8 activation provides a tempting mechanism to explain a variety of disease models in which IL-1β processing appears to be occurring independently of caspase-1.

REFERENCES

Bertrand, M.J., Doiron, K., Labbé, K., Korneluk, R.G., Barker, P.A., and Saleh, M. (2009). Immunity *30*, 789–801.

Broemer, M., and Meier, P. (2009). Trends Cell Biol. *19*, 130–140.

Feoktistova, M., Geserick, P., Kellert, B., Dimitrova, D.P., Langlais, C., Hupe, M., Cain, K., MacFarlane, M., Häcker, G., and Leverkus, M. (2011). Mol. Cell *43*, 449–463.

Krieg, A., Correa, R.G., Garrison, J.B., Le Negrate, G., Welsh, K., Huang, Z., Knoefel, W.T., and Reed, J.C. (2009). Proc. Natl. Acad. Sci. USA *106*, 14524–14529.

Labbé, K., McIntire, C.R., Doiron, K., Leblanc, P.M., and Saleh, M. (2011). Immunity *35*, 897–907.

Lopez, J., and Meier, P. (2010). Curr. Opin. Cell Biol. *22*, 872–881.

Maelfait, J., Vercammen, E., Janssens, S., Schotte, P., Haegman, M., Magez, S., and Beyaert, R. (2008). J. Exp. Med. *205*, 1967–1973.

Mao, A.P., Li, S., Zhong, B., Li, Y., Yan, J., Li, Q., Teng, C., and Shu, H.B. (2010). J. Biol. Chem. *285*, 9470–9476.

Tenev, T., Bianchi, K., Darding, M., Broemer, M., Langlais, C., Wallberg, F., Zachariou, A., Lopez, J., MacFarlane, M., Cain, K., and Meier, P. (2011). Mol. Cell *43*, 432–448.

Tseng, P.H., Matsuzawa, A., Zhang, W., Mino, T., Vignali, D.A., and Karin, M. (2010). Nat. Immunol. *11*, 70–75.

Vince, J.E., Wong, W.W.L., Gentle, I., Lawlor, I., Lawlor, K.E., Allam, R., O'Reilly, L., Mason, K., Gross, O., Ma, S., et al. (2012). Immunity *36*, this issue, 215–227.

JAK's SOCS: A Mechanism of Inhibition

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DOI 10.1016/j.immuni.2012.01.010

SOCS1 and SOCS3 are specific inhibitors for JAK tyrosine kinases. In this issue of *Immunity*, Babon et al. (2012) discovered the inhibition mechanism of SOCS3 by employing nuclear magnetic resonance and classical enzyme kinetics.

Janus kinase (JAK), which is a key signal transmitter of cytokines, has been shown to be an attractive therapeutic target for cancer and inflammatory diseases. Most cytokines, including interleukins, interferons (IFNs), and hematopoietic growth factors, activate JAKs. In mammals, the JAK family comprises four members: JAK1, JAK2, JAK3, and TYK2. JAK1, JAK2, and TYK2 appear to be ubiquitously expressed, whereas JAK3 expression is normally limited to hematopoietic cells. Activated JAKs phosphorylate the associated receptor cytoplasmic domains, which then creates docking sites for SH2-containing signaling proteins, including signal transducers and activators of transcription (STATs). The Ras-ERK and PI3 kinase pathways are also activated through JAKs. Aberrant activation of these pathways is often observed in many cancer and leukemic cells. Hyperactivation of the JAK-STAT pathway plays a role in several immunological disorders such as inflammatory diseases, autoimmune diseases, and allergy.

Several JAK2 inhibitors are under development for the treatment of myeloproliferative neoplasias (MPN) and other tumors. This is because constitutive activation of JAK2 was found in leukemias and lymphomas (via formation of chimeric proteins) and in MPN (via a V617F mutation). JAK inhibitors are also effective for tumors with constitutive JAK-STAT pathway activation without mutations. Therapeutic benefit from JAK kinase inhibition has already been established in rheumatoid arthritis (RA) with the use of CP-690,550 (Tofacitinib), a pan-JAK inhibitor. CP-690,550 was originally intended for organ transplantation immunosuppression because it is a potent inhibitor of JAK3, but has also shown to have activity against JAK1 and JAK2. More recently, the selective JAK1, JAK2 inhibitor, INCB028050, has demonstrated efficacy in various rodent models of RA, further demonstrating the central role JAK kinases play in this disease.

There is a natural inhibitor family for the JAKs: the SOCS family of proteins (Alexander and Hilton, 2004; Yoshimura et al., 2007). Overexpression of these proteins has been shown to effectively suppress tumors and RA models (Yoshimura et al., 2007). Thus, SOCS mimetics is a strategy for developing therapeutics to these diseases. However, a precise mechanism of how SOCS inhibits JAK kinase activity remains to be established. In this issue of *Immunity*, Babon et al. (2012) succeeded in obtaining NMR spectrums of JAK2 kinase domain and the SOCS3 complex, and, in combination with classical biochemical enzyme assays, they discovered an unexpected mechanism through which SOCS3 inhibits JAK kinase activity.

The suppressor of cytokine signaling (SOCS) protein family comprises eight members (cytokine-inducible SH2 protein [CIS] and SOCS1–SOCS7). The central SH2 domain determines the target of each SOCS and CIS protein. There is a conserved sequence called extended SH2 domain (ESS) adjacent to the SH2 domain, which is necessary for a high-affinity binding of the SH2 domain to the target phosphopeptides (Babon et al., 2006; Yasukawa et al., 1999). The SH2 domain (including ESS) of SOCS1 directly binds to the activation loop of JAK (Yasukawa et al., 1999). While the SH2 domains of CIS, SOCS2, and SOCS3 bind to phosphorylated tyrosine residues on activated cytokine receptors, SOCS3 binds to gp130-related cytokine receptors, including the phosphorylated tyrosine 757 (Y757) residue of gp130, the Y800 residue of IL-12 receptor β2, and Y985 of the leptin receptor, showing that suppression by SOCS3 is relatively specific to STAT3 and STAT4. SOCS3 does not inhibit IL-10-mediated STAT3